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Chapter 16
Vibrational Spectroscopy of Sub-Nanogram Samples with Tunneling Spectroscopy

by

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Capabilities, Applications and New Techniques

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Tunneling spectroscopy can be used to obtain vibrational spectra of organic compounds such as amino acids, nucleotides and hormones with samples of less than 0.7 x ~~10-10~~ grams.

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CHAPTER 16

Vibrational Spectroscopy of Sub-Nanogram Samples with Tunneling Spectroscopy

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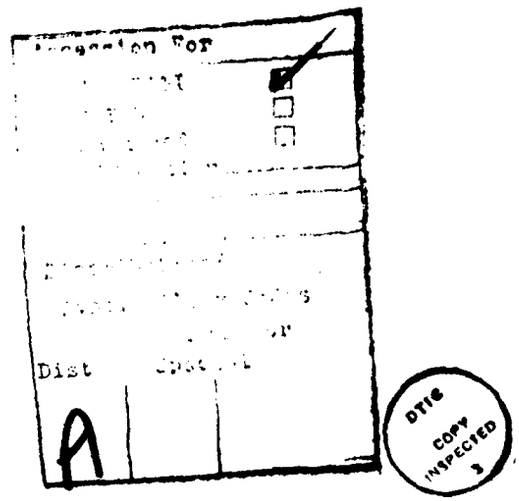

As mentioned in Chapter 1, sample preparation is the most challenging part of tunneling spectroscopy. In sample preparation, the most critical step is doping the junction. Thus, it is understandable that a great deal of time and energy have gone into a development of improved techniques for doping. The three general categories of techniques that have been discussed thus far in the book are: 1) vapor doping, 2) liquid phase doping and 3) infusion doping. In this chapter we discuss a relatively new technique for liquid phase doping in which a very small drop of dopant solution is placed on the tunneling barrier and evaporated to dryness. This simple new technique has the advantage that it requires orders of magnitude less of the doped compound than other techniques.

The first step in making the tunnel junctions is to evaporate aluminum through a stainless steel mask into strips on glass slides. These aluminum strips are oxidized by exposure to oxygen and air. The chemicals are doped onto the strips using a small platinum loop as shown in figure 1. Figure 1c is a scanning electron micrograph showing the 127 μ loop of platinum wire above an aluminum strip that is 100 μ wide and 0.08 μ thick. The platinum loop is held in the tripod just above the surface of the 1" x 3" glass slide (figure 1a). The height is adjusted with a brass thumb screw. In operation, the tripod is grasped as shown in figure 1b and moved to the end of the slide where it is dipped into a drop of stock solution containing the chemical of interest. Typical size of the stock solution drop is 1 microliter; the typical solution concentration is 0.1

grams per liter. Thus, the stock solution contains on the order of 1/10 microgram of solute. The loop is raised from the stock solution by lifting the front leg of the tripod and then is moved to the junction area. The two back legs of the tripod are pressed against an indexing strip to determine the front-back position. The lateral position is determined by eye through the magnifying glass. When the loop is approximately over the junction area, the front leg of the tripod is lowered to the slide. The drop is thus transferred from the platinum loop to the junction area.

Typically, the five junctions in a set (figure 1c) are doped with different dilutions of the stock solution by adding water to the drop of stock solution between successive pickups with the platinum loop. Finally, after all the desired junctions are doped, a drop of india ink is placed at each end of the slide with the doper. These two drops of india ink determine the line on which the dopant drops have been placed. They are used for orienting the slide over the mask when it is put back in the evaporation chamber for adding cross strips of lead metal (100 μ wide and 0.2 μ thick). The junctions are formed at the intersections of the lead and oxidized aluminum strips.

There are a few subtle differences between the experimental techniques for this sub-nanogram doping and the techniques described in Chapter 1: 1) It is valuable to use very small junction areas and very small dopant drops. Larger junction areas and dopant drops have given, in our limited experience, less reproducible results. The masks for the small metal

strips can be chemically etched to order by several firms in thin stainless steel sheets. 2) It is easy to get too high a junction resistance for these small junctions. Consequently steps must be taken to keep the resistance low. Rather than venting the chamber to pure oxygen, we vent to just 0.1 torr of pure oxygen and then vent the rest of the chamber with nitrogen or argon (venting to pure nitrogen gave unreproducible results). After the chamber is vented, we carry the slides to the doping apparatus (it takes about 20 seconds) and then at the doping apparatus maintain a gentle flow of nitrogen over the slides. 3) A convenient feature is that the platinum loop can be easily cleaned between dopants by simply holding it in the flame of a bunsen burner. It heats white hot and any organic material is burned off within a fraction of a second.

The completed junctions are treated in the same way as described in Chapter 1.

Since the concentrations of the dopant solutions are known, the amount of material transferred to the junctions can be determined if the drop size is known. The drop size can be measured by using drops of a radioactive solution. For example, we used a ^{14}C-glycine solution with an activity of approximately 500 μci/ml. We made nine drops with the loop, cut the slides with a glass cutter to separate the drops, put each into a scintillation vial with scintillation cocktail[1] and counted the vials. Standards were made by diluting the stock solution 5 μl to 500 μl and then counting 1 μl and 5 μl of

this dilute solution. The measured drop volumes were 4 ± 1 nl for the loop shown in figure 1 and 7 ± 2 nl for the (similar) loop used for making the samples of figures 2 and 3.

Figure 2 shows spectra obtained for benzoic acid solutions obtained by successive dilution of a 1 μl drop of 0.1 g/l stock solution. The top two traces were from separate junctions to illustrate the reproducibility of the technique Note that the intensity of the benzoic acid peaks (e.g. near 400 and 1600 cm^{-1}) saturates near 7 x 10^{-10} grams.

Figure 3 shows the vibrational spectra of 7 x 10^{-10} grams of compounds of current interest in biochemistry. They illustrate that the technique can be applied to amino acids, nucleotides and hormones. These compounds are also of interest as neurotransmitters.

Vibrational spectra of hundreds of other biochemicals have been previously obtained with a spin-doping technique.[2,3] It should be possible to use the current micro-doping technique with these and related biochemicals whenever one wants to use only a microliter or less of the stock solution.

It should be emphasized that these spectra of subnanogram quantities are not the minimum quantities that can be detected. In fact, quantities below a nanogram saturate the vibrational spectra. Previous evidence suggests that of order 1/100 of these saturation amounts can be detected in careful experiments.[4] Further, the loop used for these experiments made drops of order 460 μ in diameter. Though this made positioning easy in a 100 μ x 100 μ junction because of the large overlap,

it could be reduced. For example, a 200 μ diameter drop could deposit the same surface coverage with 1/5 the material. Thus, the sensitivity limit of the technique is probably below 10 picograms.

A real question is how useful is this? There are a number of problems that prevent it from being a great boon to biologists and analytic chemists, at least at present: 1) The apparatus for doing tunneling spectroscopy is not available commercially. 2) There are no collections of tunneling spectra analogous to the huge collections of infrared or Raman spectra, though the number of published tunneling spectra has been growing rapidly. 3) Typical molecules react with the aluminum oxide to form surface adsorbed species. Though these surface adsorbed species are interesting in their own right as previous chapters have shown, they are not the original parent compound, and any attempt to identify the original parent compound from the surface adsorbed species is by inference. Even if a huge collection of spectra was available, it is possible that more than one starting compound would give the same adsorbed species (for example, benzoyl chloride,[5] benzaldehyde[6] and benzoic acid[7] all give adsorbed benzoate ions). 4) The range for quantitation is very small. Spectra saturate at approximately one monolayer and become undetectable for less than 100th of a monolayer. Ideally, one would like a much wider range over which quantitative results could be obtained. Since there is no guarantee that the tunneling peak height is a linear function of the dopant concentrations, standard curves would

have to be plotted as well. 5) Vibrational spectroscopy, in general, is not good at distinguishing low levels of one compound in the presence of others. Assay techniques such as radioimmunoassay that can detect minute amounts of biochemicals in the presence of large amounts of buffers and other interfering chemicals are very much to be preferred in this regard.

For these and perhaps for other reasons, it seems unlikely that this technique will evolve into a general purpose method for analyzing trace quantities of biochemicals. This kind of prediction is, of course, dangerous, because it does remain true that despite all of its limitations, tunneling does have sensitivity to orders of magnitude less material than any other technique for vibrational spectroscopy known at present. As discussed above, the minimum quantities detectable are probably below 10 picograms (e.g. for benzoic acid, 10 picograms corresponds to 5×10^{10} molecules, 1/10 picomole or one monolayer over a square less than 1/10 mm on a side).

ACKNOWLEDGMENT

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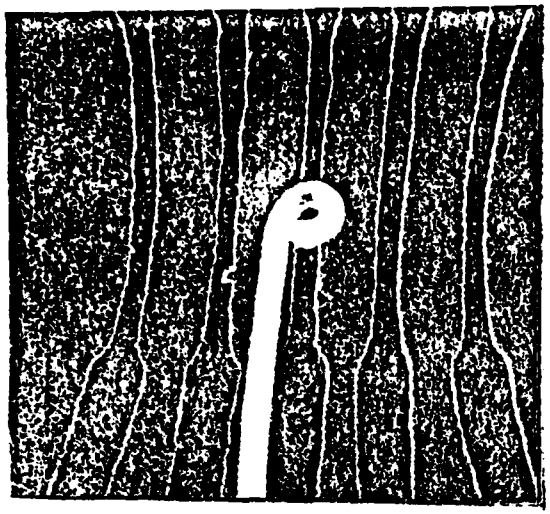
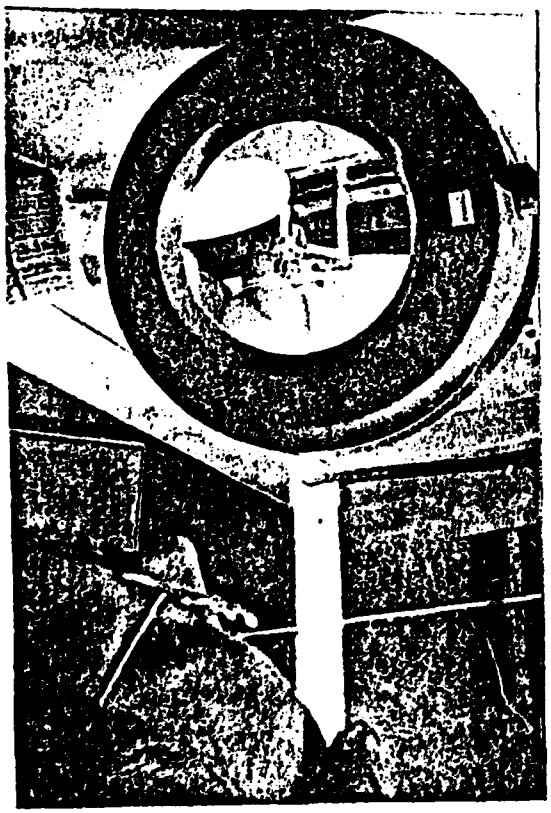
Figures - Chapter 16

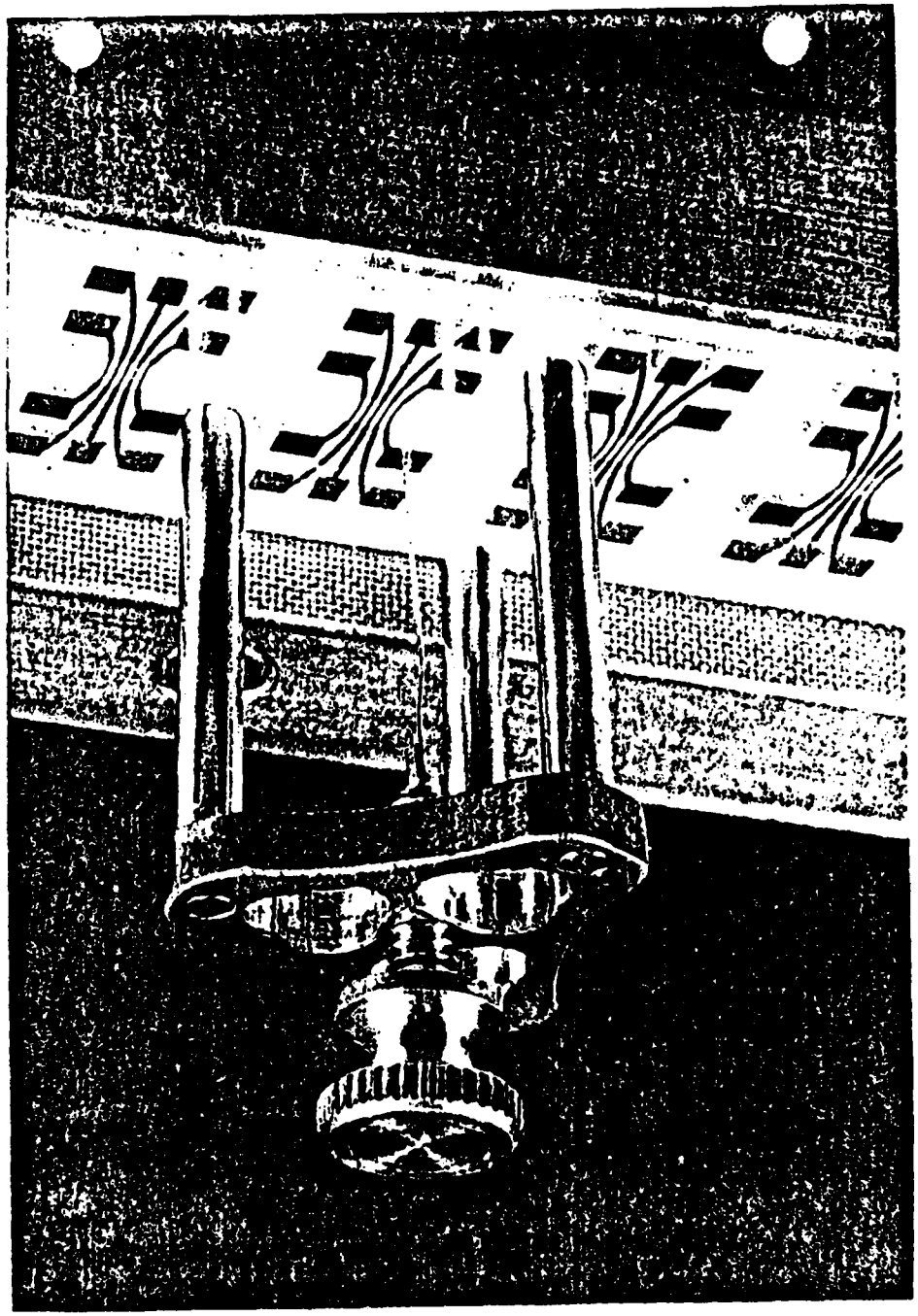
Figure 1. Apparatus and technique for applying nanoliter samples. (a) A platinum loop is held in a tripod that holds it just above the glass slide to avoid damaging the oxidized aluminum strips on the slide. (b) The loop is positioned by pushing the back two legs of the tripod against a guide rail. The loop is dipped into a microliter of stock solution at one end of the slide, then the tripod is tilted and slid on its back two legs to a position above the strip, as observed through the magnifying glass. When the third leg of the tripod is lowered onto the slide, the loop transfers a few nanoliters of liquid onto the strip. (c) A scanning electron micrograph of a loop and oxidized aluminum strips, 100 μ wide.

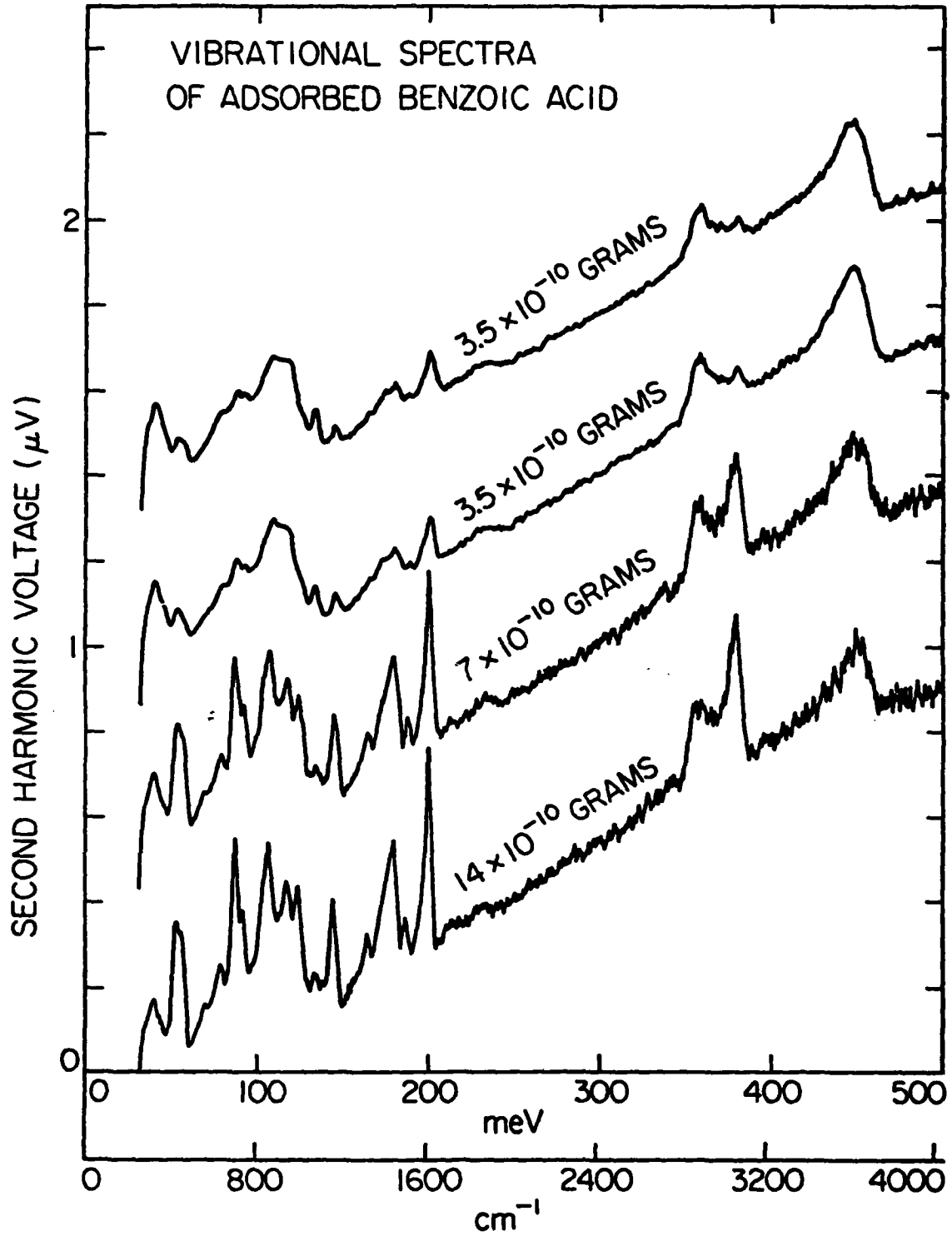
Figure 2. The doping apparatus of figure 1 was used to apply 7 nl drops of 0.05, 0.05, 0.1 and 0.2 g/l solutions of benzoic acid in water to tunnel junctions. In addition to characteristic background peaks (e.g. near 900 and 3600 cm^{-1}), there are peaks due to adsorbed benzoic acid (e.g. near 400 and 1600 cm^{-1}) that increase with solution concentration. Note the reproducibility shown by the two top curves and the saturation of peak intensities shown by the two lower curves.

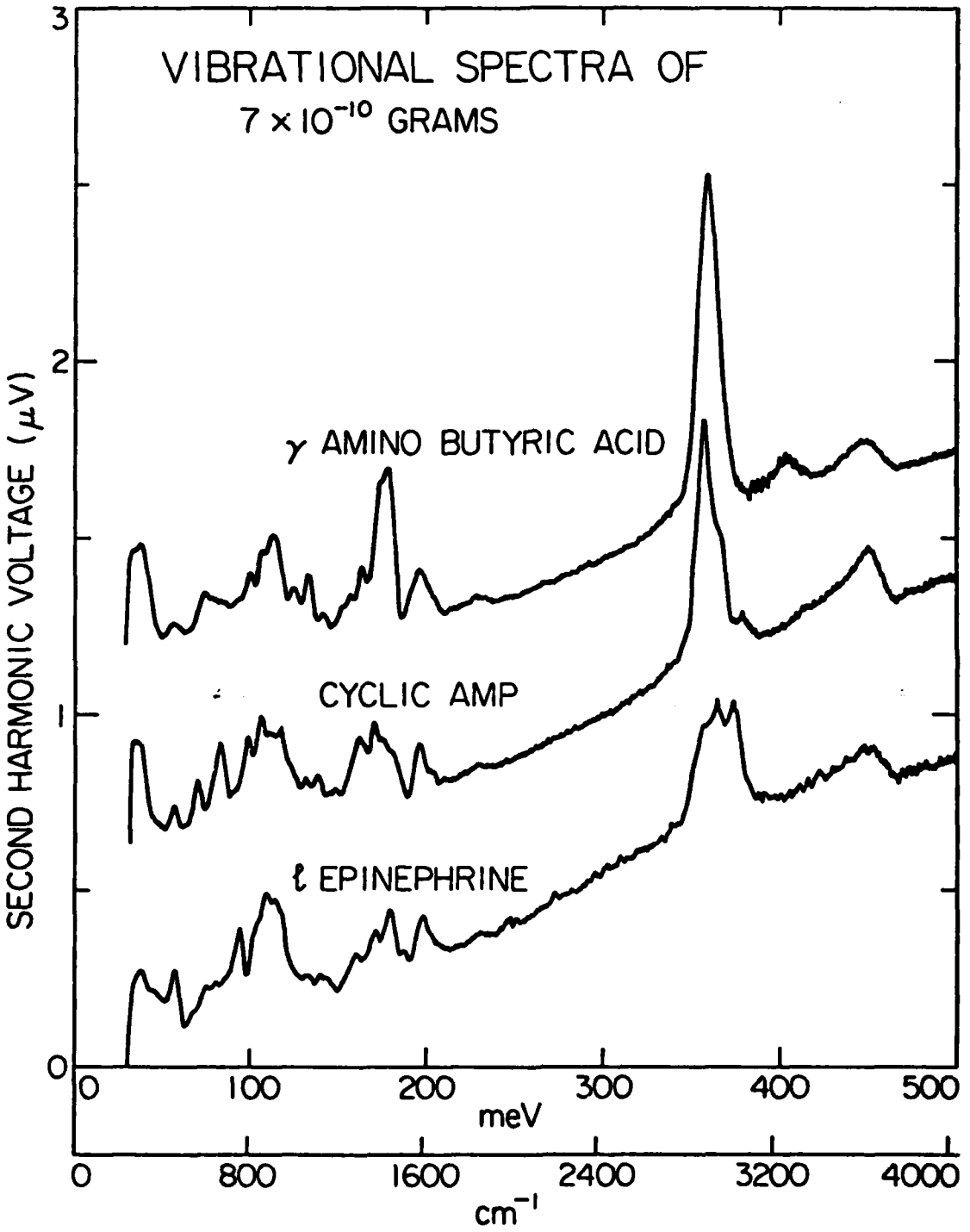
Figure 3: The doping apparatus of figure 1 was used to apply 7 nl drops of 0.1 g/l aqueous solutions of an amino acid (upper trace), a nucleotide (center trace) and a hormone (lower trace). The hormone solution had .0005 N HCl added to promote solubility and stability. This quantity of material produced a saturation of peak intensities for these compounds as shown in figure 2 for benzoic acid.

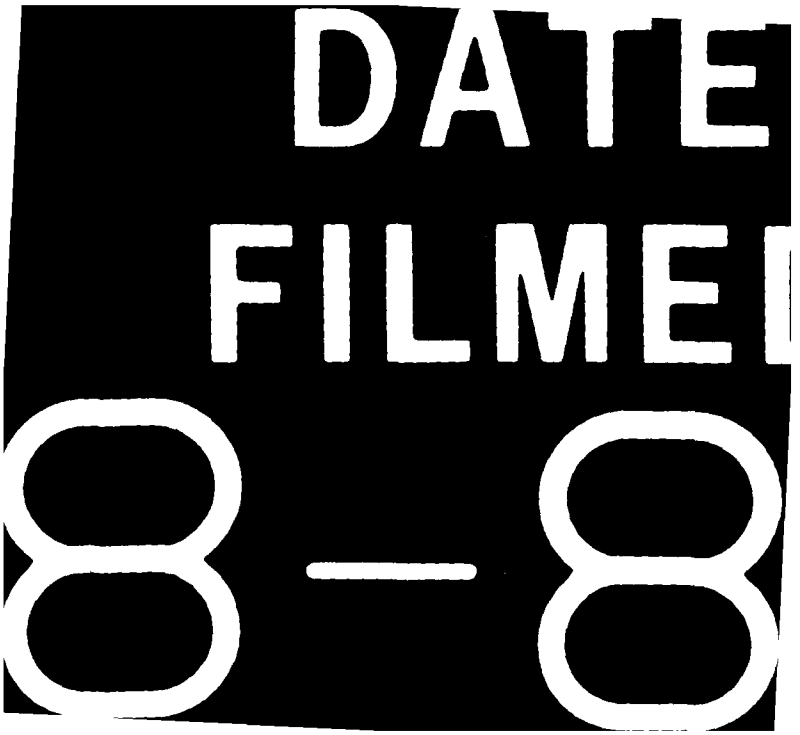







VIBRATIONAL SPECTRA
OF ADSORBED BENZOIC ACID
SECOND HARMONIC VOLTAGE (μV)
3.5x10⁻¹⁰ GRAMS
3.5x10⁻¹⁰ GRAMS
7x10⁻¹⁰ GRAMS
14x10⁻¹⁰ GRAMS
0
1
2
0
100
200
300
400
500
meV
0
800
1600
2400
3200
4000
cm⁻¹


VIBRATIONAL SPECTRA OF
7 × 10⁻¹⁰ GRAMS
γ AMINO BUTYRIC ACID
CYCLIC AMP
ℓ EPINEPHRINE
SECOND HARMONIC VOLTAGE (μV)
0
1
2
3
0
100
200
300
400
500
meV
0
800
1600
2400
3200
4000
cm⁻¹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